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Abstract

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STUDIES ON THE METABOLISM OF β-HYDROXY-ASPARTIC ACID

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Abstract: The content of β-hydroxyaspartic acid was measured in the urine of man and several species of animals. The configuration of urinary β-hydroxyaspartic acid was deduced to be L-erythro in form by chromatographic comparisons with authentic samples. An increased excretion of urinary β-hydroxyaspartic acid was observed in cats when serine or thiamine was administered with glycine. Glycine-1-^{14}C administered to rats was incorporated into the urinary β-hydroxyaspartic acid. The formation of β-hydroxyaspartic acid in pig-liver homogenate increased in the presence of glutamate and thiamine pyrophosphate. These results were discussed in relation to the author's working hypothesis on the biosynthesis of β-hydroxyaspartic acid.

β-Hydroxyasparagine has been isolated from normal human urine by Tominaga *et al.* (1). In the course of experiments in this laboratory on urinary sulfur-containing amino acids, the author noticed that β-hydroxyaspartic acid was present in fairly large amounts in the urine hydrolysates of several animal species. The metabolism of β-hydroxyaspartic acid in animals has not yet been fully investigated. The present paper examines the distribution, the carbon origin and the *in vitro* synthesis of β-hydroxyaspartic acid.

MATERIALS AND METHODS

Chemicals used were from commercial sources, including DL-erythro-and DL-threo-β-hydroxyaspartic acids (Calbiochem, La Jolla, Calif.) and glycine-1-^{14}C (Daiichi Pure Chemicals Co. Ltd., Tokyo).

Fractionation of urinary β-hydroxyaspartic acid was performed by the same procedure as reported elsewhere (2), except that hydrolysis was carried out in 2 N HCl for three hours on a sand bath, and in Amberlite column fractionation, the column was first washed with 0.2 M acetic acid (AcOH) and then eluted with 2 N HCl. As a result, the 2 N HCl eluate contained the so-called Fractions Ib and Ic [cf. Table 2 of Reference 2]. The combined Fractions IIb and IIc were also prepared. The amino acids in these fractions were determined by an automatic amino acid analyzer (Hitachi Model 034 Liquid Chromatograph).

On the analyzer, the authentic DL-threo-β-hydroxyaspartic acid yielded one peak at 37 ml of effluent and its HW-constant was 7.52, and the authentic DL-erythro-β-hydroxyaspartic acid yielded one peak at 55 ml of effluent and its HW-constant was 11.28.

On high voltage paper electrophoresis by the method of Ubuka (3), the relative mobilities of authentic samples to aspartic acid as standard (mobility, 1.0) were DL-threo isomer, 3.0, and DL-erythro isomer, 1.3. Paper chromatography in n-butanol-acetic acid-water cannot be used for the identification of β-hydroxyaspartic acids because of its very low Rf values.

The measurement of radioactivity and the quantitative determination of amino acids were carried out simultaneously by a Beckman amino acid analyzer (Model 120-B) connected with a Tri-Carb Flow Monitor System (Packard Model 3141) according to the manual. On the Beckman amino acid analyzer, DL-erythro-β-hydroxyaspartic acid yielded one peak at 92 ml of effluent and threo isomer at 62 ml.

RESULTS

1. *Urinary β-hydroxyaspartic acid concentration in humans and several species of animals and the configuration of urinary β-hydroxyaspartic acid*

As reported by Tominaga *et al.* (1), most urinary β-hydroxyaspartic acid exists as β-hydroxyasparagine. The author has determined the total amounts of free β-hydroxyaspartic acid after mild hydrolysis.

By the fractionation procedure, urinary β-hydroxyaspartic acid was collected in Fractions Ib and Ic in most cases, but sometimes it was found also in Fractions IIb and IIc. Therefore, these latter fractions were always prepared, analyzed and summed. Table 1 shows the content of β-hydroxyaspartic

TABLE 1 CONTENT OF β-HYDROXYASPARTIC ACID IN THE URINE OF MAN AND SEVERAL LABORATORY ANIMALS

Source	OH-Asp (μ moles/l)
Man	6.46-22.39
Guinea pig	1.16- 1.34
Rabbit	3.71- 7.41
Dog	19.24-31.72
Rat	22.99-27.33

Range values are shown.

acid in the urine. Rats and dogs seem to excrete a large amount of β-hydroxyaspartic acid, and guinea pigs the least among animals tested.

In amino acid analysis, β-hydroxyaspartic acid in the hydrolyzed urine overlapped authentic DL-erythro-β-hydroxyaspartic acid (55 ml of effluent). Furthermore, by paper electrophoresis, urinary β-hydroxyaspartic acid

moved at the same rate as the authentic DL-erythro isomer. When the urine was hydrolyzed in 2 N HCl for a long period, the threo isomer appeared. Urinary β-hydroxyaspartic acid may, therefore, be considered to exist in erythro form.

2. *Effect of administering glycine and other agents on urinary excretion of β-hydroxyaspartic acid*

As shown in Table 2, oral administration of glycine alone does not seem to increase the excretion of β-hydroxyaspartic acid in cats, but glycine plus serine or thiamine seems to increase the excretion to some extent. In guinea pig, however, the administration of the combined compounds did not increase the excretion of β-hydroxyaspartic acid.

TABLE 2 EFFECT OF SEVERAL COMPOUNDS ADMINISTERED ORALLY ON URINARY EXCRETION OF β-HYDROXYASPARTIC ACID IN CATS

Substance orally administered	OH-Asp excreted (μ moles/l)
None	9.3-12.9
Gly (2 g)	12.9-19.3
Gly (2 g) Ser (2 g)	36.2-36.5
Gly (2 g) Thiamine (2 mg)	51.1

Range values are shown.

3. *Incorporation of glycine-1-^{14}C into urinary β-hydroxyaspartic acid in rats*

Five rats weighing about 250 g were fed on MF solid food (Oriental Yeast Inc., Tokyo) and received per os a mixture of 0.1 mCi of glycine-1-^{14}C and 150 mg of cold glycine per day for two days, and then 150 mg per day of cold glycine for four additional days.

A total volume of about 300 ml of urine was collected from five rats during six days. The collected urine (300 ml) was first hydrolyzed in 2 N HCl for three hours on a sand bath, and the amino acids in the hydrolysate were collected by using a cation exchanger column.

The collected amino acids were then fractionated on an Amberlite IRA--68 column (150 ml of resin, acetate form) with 1500 ml of 0.2 M AcOH and then 1500 ml of 2 N HCl. The effluent and 0.2 M AcOH eluate were combined and dried, and a proper amount of the residue was analyzed on a Beckman amino acid analyzer connected with a Tri-Carb Flow Monitor System.

The 2 N HCl eluate thus obtained was dried and a proper amount was analyzed, as described earlier. The remaining 2 N HCl eluate was

further fractionated on a cation exchange column (100 ml of Diaion SK-1, H-form) with 0.5 N HCl. Each 100 ml of the eluate was dried and tested by high voltage paper electrophoresis, and fractions containing β-hydroxyaspartic acid (0–1000 ml of eluate) were combined. The combined fraction was dried, dissolved in water and adjusted to 100 ml. The solution (0.5 ml) was analyzed on a Beckman amino acid analyzer connected with a Tri-Carb Flow Monitor System. The radioactivity curve around β-hydroxyaspartic acid was reproduced on the chart of the amino acid analyzer and is shown in Fig. 1. Table 3 shows the amount and the specific activity of several amino acids excreted in 300 ml of urine. The incorporation rate of glycine-1-^{14}C into β-hydroxyaspartic acid was followed by serine.

4. *Synthesis of β-hydroxyaspartic acid in pig-liver homogenate*

Freshly obtained pig liver (50 g) was homogenized in 400 ml of 0.1 M phosphate buffer (pH 7.4). The complete reaction mixture contained 450 ml of the homogenate, 1 mmole of glyoxylic acid, 2 mmoles of glycine, 1 mmole of glutamic acid and 2 mg of thiamine pyrophosphate (TPP) in a final volume of 460 ml. The reaction mixture

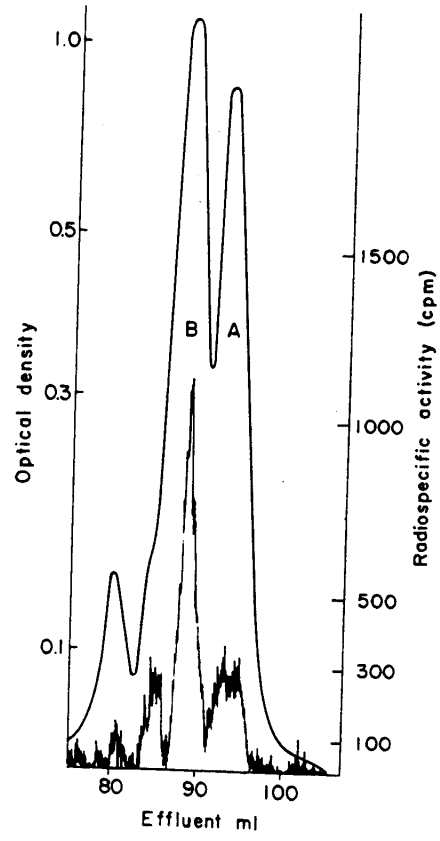


Fig. 1 Incorporation of glycine-1-^{14}C into urinary β-hydroxyaspartic acid in rats. The radioactivity curve is transposed on the figure.
A : β-hydroxyaspartic acid
B : S-(1, 2-dicarboxyethyl) cysteine

TABLE 3 VOLUME AND SPECIFIC RADIOACTIVITY OF SEVERAL AMINO ACIDS IN THE URINE OF RATS RECEIVING GLYCINE-1-^{14}C

	OH-Asp	Asp	Ser	Glu	Gly
μmoles excreted in 300 ml of urine	16.09	286.90	70.72	350.57	667.02
Specific radioactivity count/μ mole	368.11	66.92	712.67	99.84	1824.15

was incubated at 37°C for 0, 15, 30, 45, 60 and 120 minutes. After completion of incubation, 40 ml of 15% trichloroacetic acid (TCA) was added to each incubation reaction mixture and centrifuged.

The TCA supernatant was made weakly acidic with 2 N NH_3 and filtered. The combined Fractions Ib and Ic were collected from the filtrate according to the author's procedures described in the methodology section.

The combined fraction was further fractionated on a column containing Diaion SK-1 (H-form) with 0.5 N HCl. Each 100 ml of the eluate was collected, dried and tested by paper electrophoresis. β-Hydroxyaspartic acid was usually found in the eluate between 100-200 ml of 0.5 N HCl. The β-hydroxyaspartic acid containing fraction was analyzed on an amino acid analyzer.

As shown by curve ⓐ in Fig. 2A, even without added substrates, β-hydroxyaspartic acid content in the reaction mixture incubated with pig-liver homogenate increased after incubation and then slowly decreased. The same tendency was also seen in the complete reaction mixture, as shown by curve ⓑ. The reason for this phenomenon is unclear. Fig. 2B shows differences between increments of the complete reaction mixture and the homogenate only (i.e., the real increase in β-hydroxyaspartic acid).

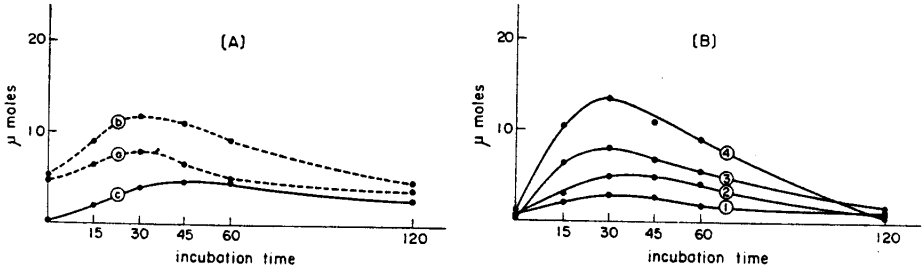


Fig. 2 Time course of β-hydroxyaspartic acid formation in pig-liver homogenate.
A: Experiments using the same pig-liver homogenate.
ⓐ homogenate only
ⓑ complete reaction mixture
ⓒ difference between complete reaction mixture ⓑ and homogenate only ⓐ
The complete reaction mixture contained 460 ml of the homogenate, 1 mmole of glyoxylic acid, 2 mmoles of glycine, 1 mmole of glutamic acid and 2 mg of TPP.
B: Four increment curves calculated by the same procedure as curve ⓒ in Fig. 2A. Each individual curve was obtained from a different pig-liver homogenate.

Table 4 shows the effect of subtraction of each substrate or cofactor from the complete reaction mixture on the synthesis of β-hydroxyaspartic acid.

In these experiments, the reaction mixture was incubated at 37°C for

30 min. The results, though not clear, seem to indicate that glutamate and thiamine pyrophosphate play an important role in the synthesis of β-hydroxyaspartic acid. The strong influence of subtracting glycine on the formation of β-hydroxyaspartic acid might be interpreted as a metabolic shift of glyoxlate to some other direction.

As shown in Table 5, alanine cannot replace glutamic acid, and aspartic acid inhibits slightly the formation of β-hydroxyaspartic acid. The latter inhibitory effect may be explained from the finding (4) that β-hydroxyaspartic acid competitively inhibits glutamate-aspartate transaminase.

TABLE 4 EFFECT ON THE BIOSYNTHESIS OF THE SUBTRACTION OF EACH SUBSTRATE OR COFACTOR FROM THE REACTION MIXTURE OF β-HYDROXYASPARTIC ACID IN PIG-LIVER HOMOGENATE

	Glyoxylate	Gly	Glu	TPP	OH-Asp formed (μ moles/1)
1	+	+	+	+	18.8
2	−	+	+	+	14.0
3	+	−	+	+	2.5
4	+	+	−	+	2.4
5	+	+	+	−	9.2
6	−	−	+	+	13.0
7	+	+	−	−	8.6
8	−	−	−	−	4.9

TABLE 5 EFFECT OF AN AMINO DONOR ON THE BIOSYNTHESIS OF β-HYDROXYASPARTIC ACID IN PIG-LIVER HOMOGENATE

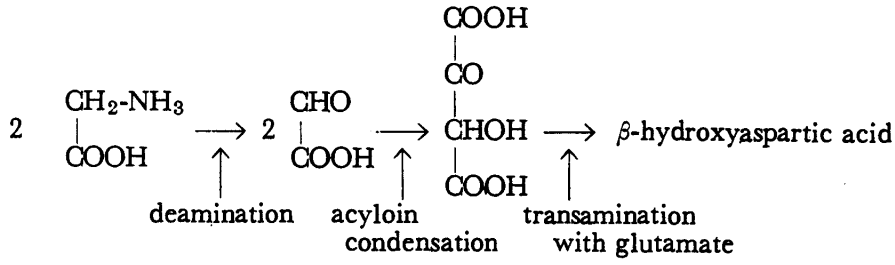
Amino donor added (2 mmoles each)	OH-Asp formed (μ moles/1)
None	4.8-5.2
Glu	5.8-9.8
Ala	2.3-2.8
Asp	0.4-1.4

Reaction mixture contained 1 mmole of glyoxylic acid, 2 mg TPP and the amino donor.

DISCUSSION

There are very few reports on the biosynthesis of β-hydroxyaspartic acid in animals. In *Micrococcus denitrificans*, β-hydroxyaspartic acid seems to be synthesized by condensation of glycine and glyoxylic acid (5). In animal tissues, β-hydroxyaspartic acid is known to be formed by a transamination reaction between glutamic acid and oxaloglycolic acid (6), althoughthe origin of the latter is not known. Under these circumstances, the author thinks

that oxaloglycolate might be formed from glyoxylate by an acyloin condensation reaction catalyzed by a thiamine enzyme. Thus, the author's working hypothesis of the reaction sequence as follow :



It has been reported that a glyoxylate carboligase found in green alga produces tartronic acid semialdehyde and carbon dioxide from two molecules of glyoxylate (7), but oxaloglycolate formation from glyoxylate by some analogous thiamine enzymes is still uncertain in other biological species.

Although definite proof for the above hypothesis has not been obtained in the present experiment, the results of the study suggest that further work along the lines of the above hypothesis will be worthwhile.

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REFERENCES

1. Tominaga, F., Hiwaki, C., Yoshida, H. and Maekawa, T.: The occurence of β-hydroxyasparagine in normal human urine. *J. Biochem.* Tokyo **53**, 227-230, 1957
2. Kodama, H., Ohmori, S., Suzuki, S. and Mizuhara, S.: New sulfur-containing amino acids in the urine of cystathioninuric patients: Supplementary data. *Physiol. Chem. Phys.* **2**, 287-292, 1970
3. Ubuka, T.: Identification of isovalthine and its related compounds. *J. Biochem.* Tokyo **52**, 440-443, 1962
4. Garcia, M-Hernandez and Kun, E.: Inhibition of enzymic transamination of aspartic acid by hydroxyaspartate, 2,3-diaminosuccinate and 2,3-diaminopropionate. *Biochim. Biophys. Acta.* **24**, 78-82, 1957
5. Konberg, L.M. and Moris, G.J.: The utilization of glycollate by *Micrococcus denitrificans*: The β-hydroxyaspartate pathway. *Biochem. J.* **95**, 577-586, 1965
6. Sallach, J.H. and Peterson, H.T.: The formation of hydroxyaspartic acid from dihydroxyfumaric acid and L-glutamic acid. *J. Biol. Chem.* **223**, 629-634, 1956
7. Badour, S.S. and Waygood, R.E.: Glyoxylate carboxylyase activity in the unicellular green alga Gloeomonase sp. *Biochim. Biophys. Acta* **242**, 493-499, 1971